IN THE WAKE OF THE MAIN PRINCIPLE BEHIND THE DESIGN OF NATURAL CYCLIC LIPODEPSIPEPTIDES

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Origin and primary structure of CLPs

Cyclic lipodepsipeptides (CLPs) are secondary metabolites typically produced by *Pseudomonas* and *Bacillus* bacterial species via non ribosomal pathways [1]. These biomolecules are constituted of a fatty acid moiety linked to the N-terminus of a peptide chain which is cyclized by an ester (or depsi) bond formation between its C-terminus and a hydroxyl group capped side chain of a Ser or Thr residue. Due to the nature of the biosynthesis, the familiar prevalence of L-amino acids is flawed by the significant presence of residues maintaining D-configuration. **The CLPs are classified depending on the length of the peptide chain and on the size of the cycle.** Herein, the eponym members of *viscosins*, *orfamides*, *xantholyisins* and *amphisins* will be scrutinized (Fig. 1). It is apparent that the **alternation of amino acids with polar and apolar side chain** is a common feature. **Is their order random or purposeful?**

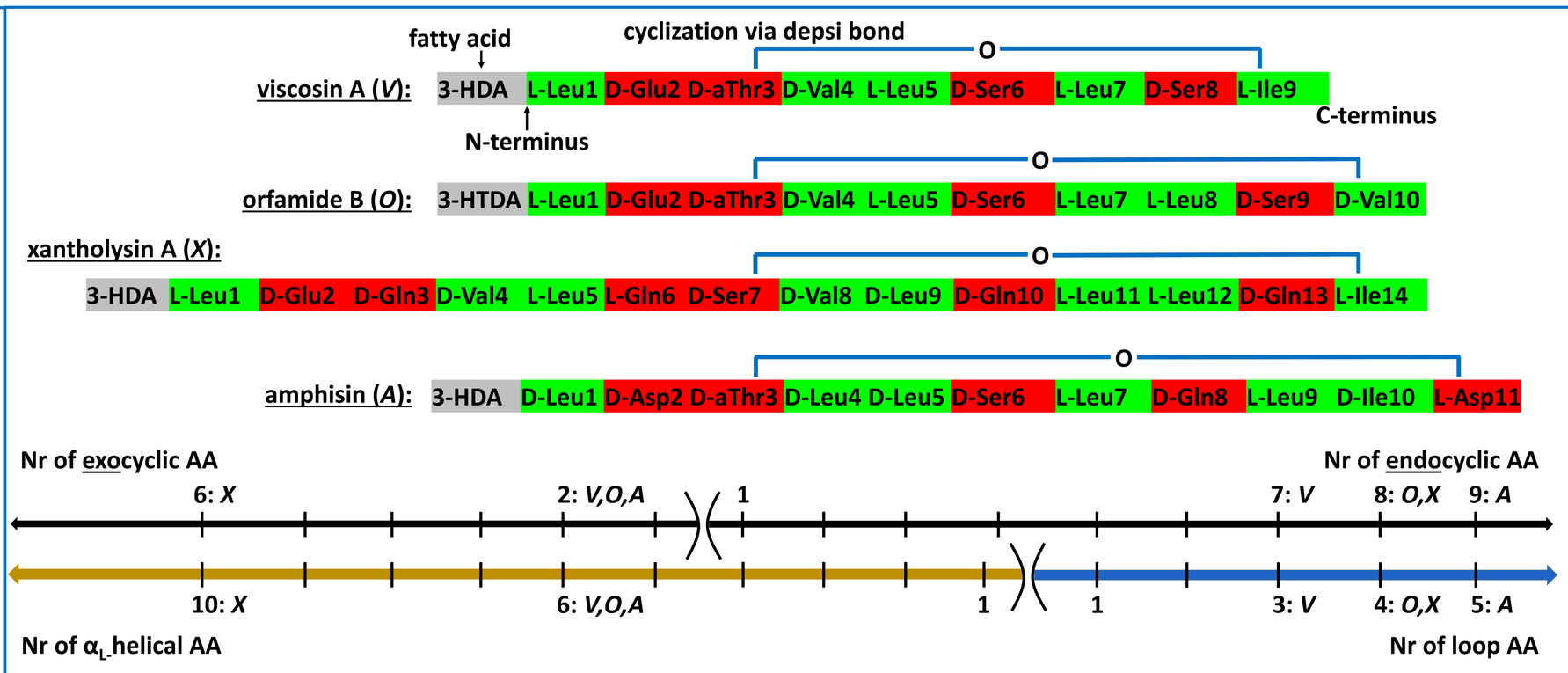

Figure 1: The primary structures of the presented CLPs. The fatty acid parts (3-HDA = (*R*)-3-hydroxydecanoic acid and 3-HTDA = (*R*)-3-hydroxytetradecanoic acid are highlighted by grey, while the amino acid residues are colored according to the polarity of their side chain, *i.e.* hydrophilic = red, hydrophobic = green.

Why CLPs? What are we looking for?

CLPs possess a wide range of biological activites: their antifungal, antiviral, anticancer and antibacterial effects [1–3] have already been exploited *e.g.* in the field of crop protection [4]. More importantly, CLPs provide either of the two classes of antibiotics that have been approved for clinical use in the past 30 years [5].

The level of bioactivity depends on the mode of action that is inherently determined by the structures of the chosen agent and its target. In case of CLPs the molecular background of the function is not well understood. To start with, **the conformation of cyclic lipodepsipeptides is yet poorly explored** (the tertiary structure is available for less than 10% of the already identified ~100 CLPs). Thus, an intention of this study is to present and correlate the previously unknown solution conformation of **viscosin A, orfamide B and xantholysin A**. Moreover, the effect of gradual ring size extension can be studied systematically by involving **amphisin** (Fig. 1) in the analysis. Such **structural comparison spanned over distinct CLP subgroups** might lead us to realize the principle behind the design of natural cyclic lipodepsipeptides which could be a milestone on the path towards the **development of novel efficient pharmaceuticals**.

How?

The peptides were biosynthetized by *Pseudomonas* strains and submitted for HPLC purification afterwards (both conducted locally, at UGent). To gain insight to the conformation at atomic resolution, **solution state NMR spectroscopy** was applied for collecting ^{1}H–^{1}H distances. This data was used as a set of distance restraints which aided the *silico* structure determination performed by **molecular dynamics simulations** using the force fields of the CNS 1.21 [6] and AMBER14 software packages [7] (Fig. 2).

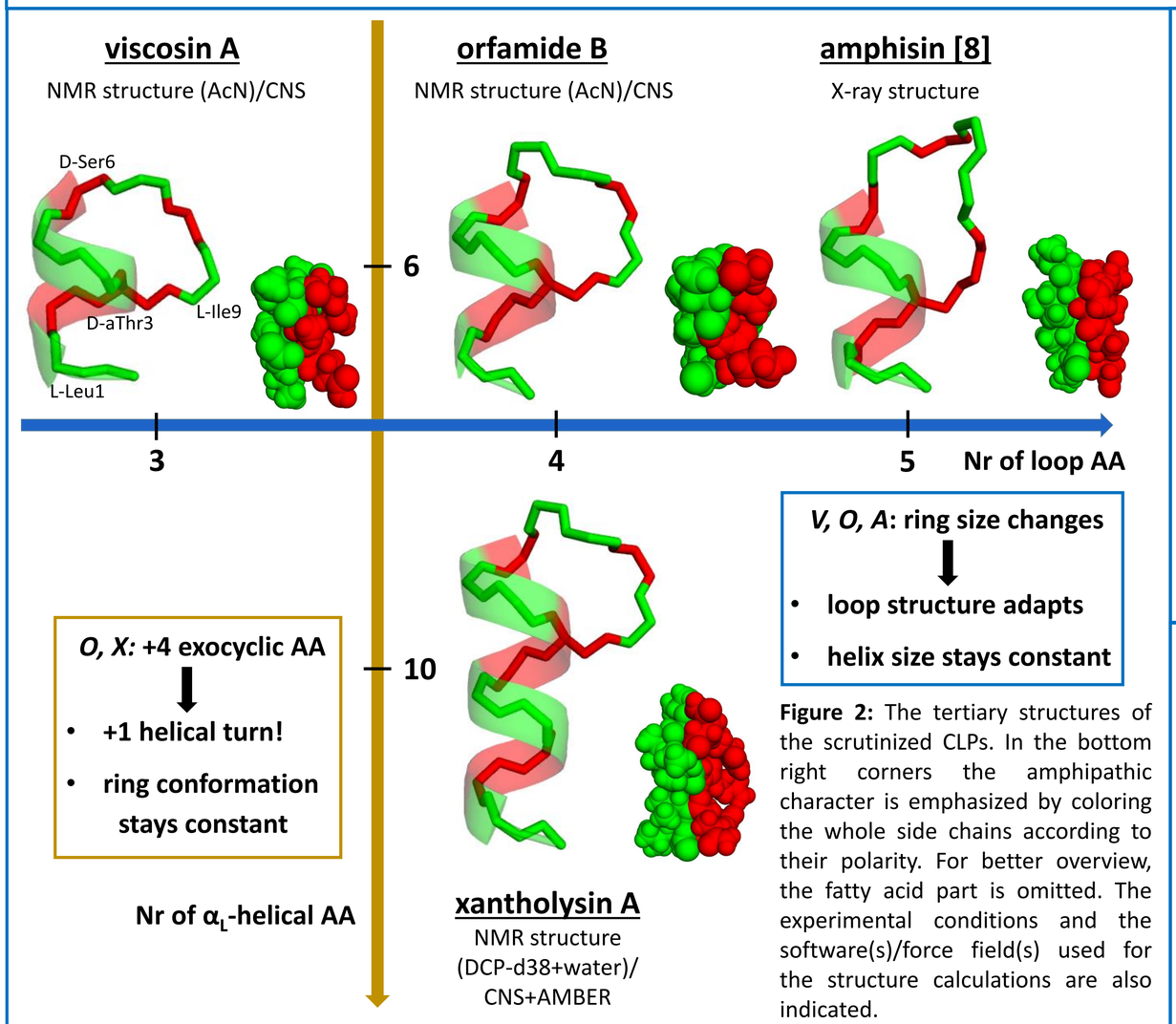

Figure 2: The tertiary structures of the scrutinized CLPs. In the bottom right corners the amphipathic character is emphasized by coloring the whole side chains according to their polarity. For better overview, the fatty acid part is omitted. The experimental conditions and the software(s)/force field(s) used for the structure calculations are also indicated.

Results – Tertiary structure

The **three-dimensional structures** of the four CLP family representatives are **built from the same elements: an α_L-helix** kicks off from Leu1 and penetrates up to the 4th endocyclic residue (Fig. 2). From then on, it proceeds into **loop** which connects the C-terminal with an OH-capped side chain via a depsi bond. This means that the length of the α_L-helix stays the same for viscosin A, orfamide B and amphisin (involving 6 residues for each) and the extension of the ring influences the loop size and conformation. The latter is varying in order to maintain the perfect separation of the hydrophobic and hydrophilic side chains what was already established by the helical motif and present for all cyclic lipopeptides in question. On the other hand, orfamide B and xantholysin A bear the same ring arrangement in terms of the side chain polarity in the respective positions. As a result, their ring and loop conformation are identical. The four extra exocyclic residues for xantholysin A simply prolong the amphipathic α_L-helix with an additional turn opposed to orfamide B. Along with the three-dimensional structures of cyclic lipopeptides containing non-natural amino acids [*tolaasins*: 2, *syringomicins*: 9], the herein presented observations confirm the **preservation of the amphipathic character** to be a fundamental driving force for the construction of natural CLPs.

Future prospects – Mode of action

Concomitantly, the insertion of the amphipathic cyclic lipopeptides in **apolar** solvent triggers their **self-assembly**. The phenomenon was examined in detail for pseudodesmin A (member of *viscosins*) which pointed out the occurence of **tubular-shaped associations** (Fig. 3) [10]. A familiar explanation for the mechanism of action of antimicrobial peptides is the creation of such pore-like associations which **disrupt the plasma membrane** and cause the lysis of the cell [11]. Obviously, investigating the bioagent alone does not allow to make general assumptions, since the actual process depends on the chosen target as well.

The application of the **^{15}N and ^{13}C labelled** version of the **peptides** would enable to demonstrate the presence and **distinguish between inter- and intramolecular H-bonds** [12], thus to reveal the structure of self-assemblies or even coaggregates of peptides and membrane mimicking detergent micelles/bicelles at high resolution. Such experiments could take us one step closer to the revelation of the structure–function relationship of CLPs. As the recent efforts had shown, growing viscosinamide-producer *Pseudomonas sp.* DR54 in a minimal salt medium prepared by the addition of $^{15}NH_4Cl$ successfully yielded the ^{15}N-enriched biomolecules in interest, so this method is planned to be accomplished the isotopical labelling of other CLPs as well.

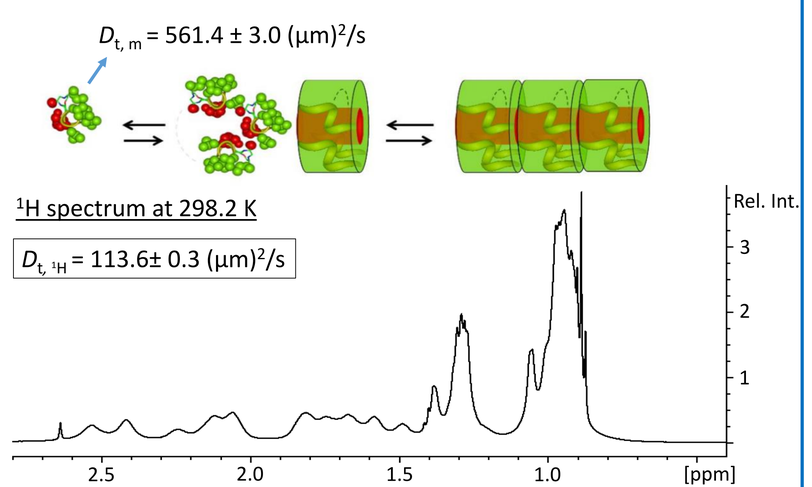

Figure 3: Top: the proposed model for the self-assembly based on diffusion measurements (adapted from [10]). Bottom: the peaks in the ^{1}H spectrum of pseudodesmin A dissolved in $CDCl_3$ (15 mM) care uniformly broadened due to the hindered rotational diffusion. Here, only the aliphatic region is displayed. The translational diffusion constant measured for the assembly ($D_{t,\,^1H}$) and the hypothetical value for a monomer ($D_{t,\,m}$) are also displayed.

Conclusion

By systematically studying the effect of the total peptide chain and/or ring residue number on the overall conformation, we can conclude that the amphipathic character is a crucial factor in the design of natural CLPs, irrespectively of the exact amino acid composition. The ^{15}N and ^{13}C labelled lipopeptides will make the direct identification of intra- and intermolecular H-bonds in self-assemblies/peptide–detergent coaggregates feasible, thus bring more details to light about the mode of action behind the versatile bioactivity of these biomolecules.

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